INSTRUCTIONS FOR COMPLETING THE NIMH ANIMAL STUDY PROPOSAL (ASP) FORM

The Animal Welfare Act and the PHS Policy on Humane Care and Use of Laboratory Animals requires review and approval of an ASP by the Animal Care and Use Committee (ACUC) prior to initiation of any activity involving vertebrate animals.

GENERAL INSTRUCTIONS:

- Form must be typed. Submit **11** copies, as well as the original before the close of business the last day of each month.
- Fill in ***each*** space. Incomplete or illegible protocols will be returned for completion before processing. All information in this ASP is considered privileged and confidential by the ACUC and the concurring authorities; however, any approved ASPis subject to release under the Freedom of Information Act.
- Copies of all appropriate guidelines are available from the Veterinary Medicine and Resources Branch, (VMRB) NIMH, Bldg. 36, Rm. 1A27; 496-4813.

SECTION A: ADMINISTRATIVE DATA:

- Principal Investigator: Only one person (the principal investigator) involved in a study must assume all responsibility for the ASP and it's execution.
- Project Title: Avoid terms that may elicit negative attention to your ASP.
- Submitted ASP: Indicate whether this is an initial submission, renewal or modification of the ASP. If this is a renewal or modification, cite the original proposal number. Modifications of ASPs must be submitted for ACUC review and approval for any significant changes in the originally described procedures.
- Personnel: A list of all personnel who will be conducting procedures involving **live** animals under this ASP.

SECTION B: ANIMAL REQUIREMENTS:

- Identify the species, age/weight/size, sex and the strain/stock designation of the animal. A separate ASP must be submitted for each species or strain unless more than one species or strain is used for a single experiment (1.e., strain comparisons, or xenotransplantation). In such cases, explain on separate sheet.
- Indicate proposed location for animal holding and procedures (building and room).
- Indicate the number of animals to be used during each of the 2 years of the approval period and the total number of animals to be used.

SECTION C: TRANSPORTATION:

Copies of the NIH Transportation Guidelines and other applicable facility transportation documents are available from the VMRB, from the NIH Office of Animal Care and Use (65424), or the training manual guidelines (Tab VIII and IX).

SECTION D: STUDY OBJECTIVES:

Briefly explain in non-technical terms the objective(s) of the study. Specifically explain what are the probable benefits of this work to human or animal health, the advancement of basic knowledge, or the good of society. A lay reader, should, by reading this, get a sense of why you want to do the study and why it is worthwhile.

SECTION E: RATIONALE FOR USE OF ANIMALS:

E1: Why is animal use necessary to attain the study objectives described in D? If necessary, state the obvious. Examples of how to address the rationale for animal use are given below:

- The investigation of the localization and amounts of mRNAs for cytokines and their receptors in brain requires the use of a living animal with a well-developed central nervous system.
- The investigation of the effects of anterior hypothalamic lesions on immune responses can only be performed on living organisms with well-defined and intact nervous and immune systems.
- The use of animal cells in culture is the most efficient method to study the neurochemical processes of interest to us. Other options would require greater numbers of animals and survival surgeries.
- The question being addressed in this proposal requires the use of highly developed animals because of the complexity of the neural connections of interest.
- Live animals are necessary to study the effects of neuronal input on neuropeptide gene induction on endocrine organs as synthetic equivalents of innervated endocrine organ do not exist.
- A fully functioning animal with well-developed nervous and immune systems are necessary to investigate the effects of stress on the neuroimmune system.
- Living animal cells are necessary to study the translation of exogenous mRNAs. Oocytes have been used very successfully for these types of studies.
- This study depends on complex behavioral functions that require a functioning animal with a highly developed nervous system.
- To our knowledge, there are no non-animal alternatives to the neural and behavioral research proposed.

E2: Describe the biological characteristics of the species listed that are essential to the study. Include experience with the proposed model and manipulation. Are other animals suitable? Why can't a lower order mammal, invertebrate, etc. be used? (This is especially important for large animals, primates, and carnivores.) Previous accumulation of data is an appropriate justification for the use of the species in the study.

E3: Explain the number of animals proposed for use in this study (the numbers of animals listed in Section B). Describe how you arrived at the number of animals requested and why that many are required. Specifically address why fewer animals cannot be used. You may wish to use one of the following three approaches in your explanation. In some cases, none of these approaches will be the most appropriate, and it may be necessary to use another approach to explain efficiently the number of animals required. Some statement should be made as to why the "n" described was chosen. It is equally important to show that enough animals are being used to generate statistically valid data as it is to show that the number proposed is not so large that animals are wasted. Guidance for animal numbers justification outlines 3 different approaches to the justification provision:

- If the experimental design dictates the number of animals required —

  The explanation should indicate and explain the numbers and types of groups, the numbers of animals per group, and the total number of experiments to arrive at a total number of animals. Factors which may reasonably be expected to change the number of animals in a significant way should also be included to preclude future amendments.
  Example: We estimate that 200 animals will be necessary for this study because we will be using five (5) animals per group (the minimum number needed to attain reliable results), and examining the effects of five (5) compounds (including vehicle), at four (4) doses of drug per compound, with one (1) replication per determination. (5 x 5 x 4 x 2 = 200).

- If the use of animals is for the harvesting of normal tissues, organs or fluids for in vitro use —

  A short statement citing past usage levels per year (per experiment, etc.) necessary to meet the tissue or fluid requirements for the study would be sufficient. If no prior experience is available, a statement relating anticipated amounts of material needed and the number of animals expected to provide that amount would be sufficient.
  Examples: The 200 animals listed is based on our experience and reflects the number required in our judgment to generate a number of cultures adequate for the anticipated rate at which assays will be done in the lab over the next two years.
  We estimate that four (4) animals will be required to generate the 50 mg of material needed for each assay. Since we perform approximately one assay per week, we estimate that 208 (4 x 52 = 208) animals per year will be required for these studies.

- If animals are used for the production of biologic reagents or cells, i.e. antibody production, tumor propagation, etc. —

  Give a simple explanation (based on experience or anticipated production capability) of how many animals would be necessary to provide a volume or other measure of material necessary for the study. This should also be related to the number of antigens, tumors, etc. that are necessary for the proposal.
  Example: We anticipate generating ten (10) neuropeptide antisera which generally requires three (3) animals per antigen. We therefore estimate that 30 (3 x 10 = 30) animals will be required for these studies.

SECTION F: DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:

This section should include a "complete" description of the proposed use of the animals. The reader should be able to visualize each procedure as it will be performed. Explain the experimental design and procedures as they affect the animals. Experimental endpoints must be used to define when experimental animals are to be euthanized or provided therapeutic relief. If it is not obvious, state how these procedures and endpoint are needed to accomplish the objectives as described in Section D. In vitro procedures need not be described at length. Details of each procedure impacting on the pain/stress/distress potential of the procedure should be specified. Use the details listed in the ASP form for guidance on the type of information, and the detail, that should be included here. For drug studies it is not necessary to anticipate every specific drug and dosage that will be used. However, list representative drugs, dosages, dose schedules (if applicable), and the route of administration for each category of drugs to be used in each type of experiment. (Anesthetic regimens should be described under Section I). For repetitive procedures (such as training, testing, etc.) indicate the time course of the experiment (i.e. describe both the lengths of the individual sessions and the anticipated time schedule for the completion of all sessions. Where appropriate, cite that the relevant NIH guidelines will be followed. (Copies of NIH/NIMH guidelines are available from the VMRB.) Elaborate on the use of any hazardous agents in Section K. Elaborate on special concerns/requirements in Section M.

SECTION G: SURVIVAL SURGERY:

Survival surgery : a surgical procedure from which an animal is allowed to recover from anesthesia.
Major survival surgery: any surgical intervention that penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover.
Non-survival surgery: a surgical procedure in which the animal is euthanized prior to recovery from anesthesia.

For example, injection or insertion of a microelectrode or needle is not surgical penetration.
Cannula insertion and subcutaneous implants are not considered MAJOR survival surgery.

1. Describe the surgical procedures to be performed, including aseptic techniques utilized. If using rodents, stating that the NIH Rodent Surgical Guidelines will be followed is sufficient to describe aseptic technique.
2. Indicate who will perform the surgery and their qualifications or experience to perform the procedures listed.
3. Specify the Building and Room number where survival surgeries will be performed. Specialized surgical facilities are required for survival surgical procedures proposed in rabbits and higher species such as cats, dogs, and primates. Surgery on rodents should be performed in suitably prepared areas outside of animal holding rooms.
4. List requirements for post-operative care and identify the person responsible for this care. Include any needed supportive therapy (the animal will be kept warm, IV fluids administered, etc.); observations necessary to evaluate the animals' health (observation of the animal, the surgical site, etc.).
5. Answer yes or no according to whether major surgery has been performed on any animal prior to being placed on this study, and if so, explain (Example: When operated animals are obtained from a vendor, state what procedure has been done and the name of the vendor performing it.)
6. Multiple major survival surgical procedures on a single animal are discouraged and may only be performed if they are a justifiable requirement of a specific research project, or if they are required as routine veterinary procedure (as determined by the clinical/facility veterinarian). The need to perform multiple major survival surgical procedures must be scientifically justified in this Section.

SECTION H: PAIN OR DISTRESS CATEGORY:

Indicate the number of animals to be used per year in each category. These numbers should be in agreement with those given in Sections B and E. The definitions and examples in Attachment 1 should provide guidance in completing this section appropriately. Category E procedures are included in the proposal, a separate page should be attached which details why these procedures are unavoidable in order to achieve the experimental goal. The restraint of unadapted animals by chair or stock for a period of more tha 12 hours that causes unrelieved pain or distress for that animal requires a Column E justification and must be included in the Anim Study Form.

SECTION I: ANESTHESIA, ANALGESIA, TRANQUILIZATION:

List the types, doses, and routes of administration of anesthetic, analgesic, and/or tranquilizer to be used. The schedule or indications for administration should be provided. If agents are to be given "as needed," a brief description of the indications for its administration should be provided (e.g. "at the first indication of discomfort as evidenced by...") It is recommended that investigators consult a veterinarian when choosing anesthetics to ensure that the agent, dose, and route of administration is appropriate for the species and size of animal being used.

SECTION J: METHOD OF EUTHANASIA:

- Indicate method used and, if a chemical agent is used, specify dosage and route of administration. Methods of euthanasia which are not consistent with recommendation of the 1993 AVMA Panel Report on Euthanasia must be scientifically justified. If decapitation or cervical dislocation without anesthesia is proposed, provide scientific justification.
- If the animals are euthanized as a part of the study, state how the carcasses will be disposed of. If the animals are not going to be euthanized as a part of the study , specify the disposition of the live animals (e.g. "Animals will be returned to the veterinary section for reissue at the conclusion of the study...").

SECTION K: HAZARDOUS AGENTS:

This section must be completed if hazardous agents will be used in animals. The use of hazardous agents requires the concurrence of the safety representative (see Section O). Refer to all appropriate safety documents (and attach as necessary) as specified in this section (e.g., radiation safety protocols, Recombinant DNA documents, etc.) in consultation with the appropriate safety officer.

- Radionuclides: Identify radioactive isotopes used and their activity. The Radiation Safety Branch's Health Physicists' signature required (see Section O).
- Biological Agents: List viruses, bacteria, and any blood or body fluids potentially infectious to humans. A Human Pathogen Registration document must be filed with the Occupational Safety and Health Branch (OSHB) for the use of these biologicals. Identify the registration document number. Safety Specialist signature required (see Section O).
- Hazardous Chemical or Drugs: List any hazardous chemicals or drugs (carcinogens, mutagens, etc.). Safety Specialist signature required (see Section O).
- Recombinant DNA: Identify any nonexempt Recombinant DNA made in the course of this study. A recombinant DNA registration document must be filed with the NIH. Safety Specialist signature required (see Section O).

SECTION L: BIOLOGICAL MATERIAL/ANIMAL PRODUCTS:

Biological material and animal products (cell lines, tissues, and tumors) destined for use in microbiologically defined rodent facilities must be MAP, RAP, or HAP (Mouse, Rat or Hamster Antibody Production) tested prior to introduction unless they are sterile or attenuated. A copy of the test results should be attached to the ASP. NCRR/VRP must approve prior to the introduction of any rodent, rodent product, or biologicals that may harbor agents of animal diseases considered dangerous to the programs of the NIH. NIH Form 2369-1, *Application for Permit to Introduce Rodents and Rodent Products*, must be submitted through the ICD Veterinarian for approval before animals or animal products from unapproved sources are introduced into any NIH or ICD facility.

SECTION M: SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY:

List any items which may be of special concern. Procedures of concern include those involving chairing or other restraint, food, or water restrictions, stress, etc. Specify the restraint, food and/or water restriction or stressor to be used and the rationale and scientific justification for their use. The NIH has policies which address many of these special concerns. It may be useful to refer to the relevant policy here. List any special housing, animal care, or safety requirements. List any other requirements that the animal facility manager may need to consider. Potential concerns include importation of animals, specialized housing, lighting, feed, or water; a need for other than routine veterinary care; use and storage of specialized pieces of equipment; special off-hour or weekend/holiday requirements. If animals are necessarily held in a laboratory for more than 12 hours, it should be described (and justified) here.

SECTION N: PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. Do not leave the Year of Course Attendance blank. If you need the date of your course attendance, or wish to enroll in the next available course, contact your ICD veterinarian's office prior to submission of this form.
2. You are signing to certify that this is true.
3. If information or assistance is necessary for enrolling in the Animal Exposure Surveillance Program (AESP), contact your ICD veterinarian's office.

4. You are signing to certify that this is true.
5. If all of the proposed procedures are listed in Column C of Section H of the ASP, this item need not be completed. Examples of methods and sources used to complete the search to certify that alternatives to the PROCEDURES proposed in this study are not available include:
    - Databases searched
    - Pertinent reference, bibliographies, or other publications
    - Information services utilized (Examples of these services and how to contact them is available from your ICD veterinarian's office.)
    - Consultation with individuals having expertise in the field of investigation.
6. **All proposals must be signed by the P.I.** Your signature below certifies, among other things, that you will inform the ACUC in writing of any significant changes in your proposed studies. All changes which may have an impact on animal welfare or policy issues, including but not limited to surgery, food or water restriction, restraint, and induction of stress **must be approved by the ACUC prior to initiation**. However, ACUC approval is not necessary for minor procedural changes within NIH policy. It is recognized that some procedural details cannot be reliably anticipated and changes in planned procedure are often dictated by findings emerging during the course of a project. If you need to make a procedural change and are uncertain whether you should report the change to the ACUC, consult your ICD veterinarian's office or an ACUC member. All changes in personnel must be reported to the ACUC.

SECTION O: CONCURRENCES:

It is the responsibility of the Principal Investigator to obtain the signature of the Lab/Branch Chief. Proposals originating from a Lab/Branch Chief require the concurring signature of the Scientific Director. The signature of the pertinent Safety Officer, Facility Veterinarian, etc. should be obtained by the Principal Investigator, where applicable, prior to ASP submission.

SECTION P: FINAL APPROVAL:

Self-Explanatory.

USDA GUIDELINES FOR PAIN/DISTRESS CLASSIFICATION

(for guidance only in categorizing animal use)

The following definitions and examples are proposed as a means for obtaining uniformly consistent USDA reporting by each of the ICD's of NIH. Even though such information is required by law, it is the intent of these Guidelines to foster exemplary care independent of legislation.

Definitions

Pain is awareness of acute or chronic discomfort occurring in varying degrees of severity resulting from injury, disease, or emotional distress and evidenced l biological or behavioral changes or both.

Acute Pain results from a traumatic, surgical, or infectious event that is abrupt in onset, relatively short in duration, and generally alleviated by analgesi Associated distress may be responsive to tranquilizers.

Chronic Pain results from a long standing physical disorder or emotional distress that is usually slow in onset, has a long duration, and is generally n totally alleviated by analgesics, but frequently responds to tranquilizers combined with environmental manipulation and behavioral conditioning.

Distress is undesirable physical or mental stress resulting from pain, anxiety, or fear. Its acute form may be relieved by tranquilizers, while sustain distress requires environmental change and behavioral conditioning, and does not respond to drug therapy.

The following examples are offered to provide further clarificaitons and guidance:

Column C - Minimal, transient, or no pain or distress

These procedures are considered to produce minimal, transient, or no pain or distress when performed by competent individuals using recognized methods.

1. Administration of:
   a. Anesthetics, analgesics, and tranquilizers
   b. Fluid and electrolyte therapy
   c. Immunizations
   d. Oral medications
2. Non-chronic catheterization
3. Blood collection (except intracardiac, and periorbital in some species, see below)
4. Gastric gavage
5. Certain procedures performed in the normal practice of veterinary medicine and those involving the diagnosis and treatment of disease (e.g., injections, palpations skin scraping, radiography).
6. Euthanasia as performed in accordance with recommendations of the AVMA Panel on Euthanasia.
7. Intracerebral inoculations in neonatal rodents. In many neonatal rodents intracerebral inoculations can be performed by trained personnel prior to cranial ossification, producing only transient pain or distress.

If the result of any of the above procedures is painful or distressful the procedure should be listed under Column D or Column E below.

Column D - Pain or distress relieved by appropriate measures

Examples of procedures that may produce pain or distress, but which are performed using appropriate and adequate anesthesia, analgesia, or tranquilization a followed with appropriate measures to alleviate pain or distress are as follows:

1. All surgery, including biopsy, gonadectomy, and neurophysiological manipulations or preparations such as implantation of electrodes and recording devices, and alterations to nerve or muscle fibers.
2. Burning, freezing, and branding.
3. Fracturing bones
4. Electrical shocks including shock reinforcement, using voltage which is accepted as generally causing pain in humans.
5. Use of any agent that induces excessive inflammation or necrosis.
6. Drug or radiation toxicity testing, including LD50 determinations, producing pain and distress.
7. Skin or corneal corrosivity testing, including Draize testing.
8. Intracardiac blood collection.
9. Periorbital collection of blood from any species except mice and hamsters. Note: Periorbital collection from unanesthetized animals that do not possess a true orbital sinus, as do mice and hamsters is discouraged.

Column E - Unrelieved pain or distress

Procedures, including those listed above for column D, which are performed without appropriate and adequate anesthesia, analgesia, or tranquilization or which are not followed with appropriate measures to alleviate pain or distress, or which are not amenable to relief by therapeutic measures, must be listed in column E and must be justified.

1. An LD_{50} study testing a toxic chemical, infectious agent or radiation exposure may cause illness and eventual death in many of the higher dose animals of the experimental group. Although some of these animals may recover from serious effects of the exposure, they should all be listed in Column E. Other, lower dose animals in this experimental group, may suffer only minor illness, or no illness at all. These animals should be listed in Column C. An investigator testing an agent that causes an immediate loss of consciousness and a rapid painless death would list all animals in Column C. (See the IRAC Recommendation on LD_{50} Testing.
2. The restraint of unadapted animals by chair or stock for a period of more than 12 hours that causes unrelieved pain or distress for that animal requires a Column E justification and must be included in the Animal Study Proposal.

BEHAVIORAL AND CLINICAL SIGNS OF PAIN*

A. Behavioral Signs

Species	Posture	Vocalization	Temperment	Locomotion	Other
Rat Mouse	Persistant doormouse posture	Squeals on handling or pressure on affected area	May become more docile or aggressive		Abdominal writhing in mice; eats bedding; eats neonates
Rabbit	Looks anxious, faces back of cage (hiding posture)	piercing squeal	Kicks and scratches	or dozy	No spillage of food or water; eats neonates
Guinea Pig		Urgent repetitive squealing	Rarely vicious; usually quiet; terrified, agitated	Draws back legs	No spillage of food or water
Dog	Anxious glances; seeks cold surfaces; tail between legs; "Hangdog" look	Howls, distinctive bark	Aggression or cringing and extreme submissive-ness; runs away		Penile protrusion frequent urination
Cat	"Tucked in" limbs hunched head and neck	Distinctive cry or hissing and spitting	Ears flattened; fear of being handled; may cringe		
Monkey	Head forward, arms across body	Screams	Facial grimace		

B. Clinical Signs

Species	Cardiovascular	Respiratory	Other
Rat**	Dark claws and feet, eyes bulge	Shallow, rapid breathing; grunting on expiration	Red staining around eyes and nose; cyanosis; congestion and jaundice in mucus membranes or non-pigmented and non-hairy areas, Square tail (dehydration)
Rabbit		As rat	White discharge from eyes, nose and on inside forepaws; cyanosis, congestion and jaundice in mucus membranes, or non-pigmented and non-hairy areas.
Guinea Pig		As rat	Cyanosis, congestion and jaundice in mucus membranes or non-pigmented and non-hairy areas
Dog		As rat, salivation and panting	As guinea pig. Raised body temperature; increase in urine Specific Gravity and decrease in volume; sweaty paws, pupils dilate, eyes glazed
Cat		As dog	As dog. Circumanal gland discharge, third eyelid may protrude
Monkey		As dog	As dog*

Modified from Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Morton, D.B. and Griffiths, P.H.M. The Veterinary Record, April 20, 1985.

** Many signs in rats may also be seen in mice

EXAMPLE ALTERNATIVE ENDPOINTS FOR STUDIES WITH POTENTIAL LETHALITY

Alternative Endpoint	Example	Application
Tumor Characteristics	10% of normal body weight, necrosis, infection	subcutaneous or intraperitoneal tumors and hybridomas
Peripheral Blood Cell Counts	Depends on cell type	Leukemias, infectious disease, anemia
Prolonged Inappetence/Cachexia	Loss of weight (20% of normal body weight) and/or condition	Metastatic disease, chronic infectious disease
Inability to Obtain Feed and Water	Paralysis, orofacial or cervical lesions, other non-ambulatory condition	Many
Signs of Severe Organ or System Involvement	Respiratory: rapid or labored breathing coughing, rales	Toxicity testing
	Cardiovascular: Severe diarrhea or vomiting	
	Peripheral Nervous System: flaccid or spastic paralysis	
	CNS signs: circling, blindness, dementia, convulsions	
	Integument: extensive hair loss, inflammation	
Moribund or Pre-moribund State	Define with specific clinical signs and euthanize when reached	Many